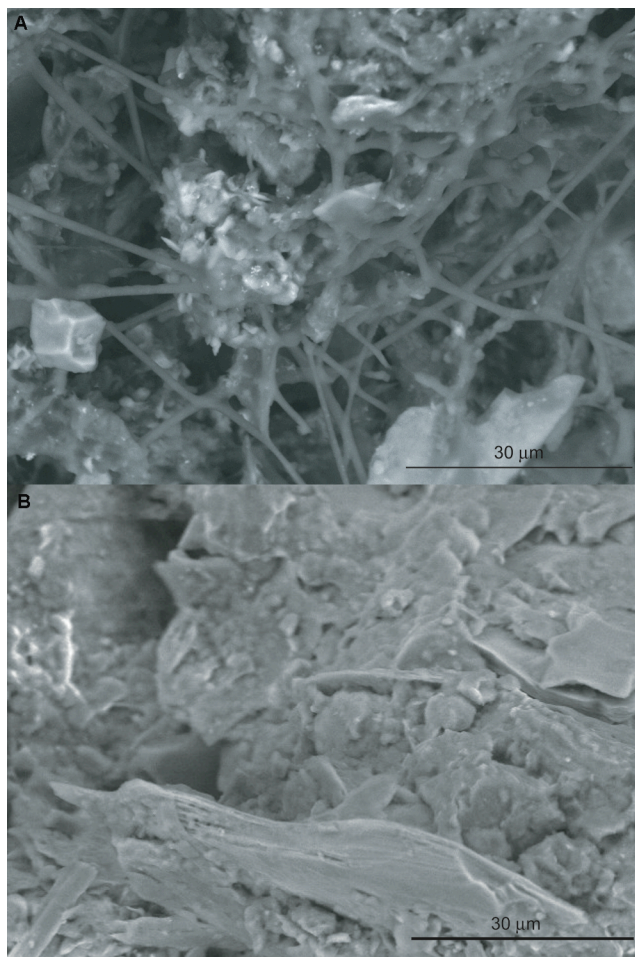
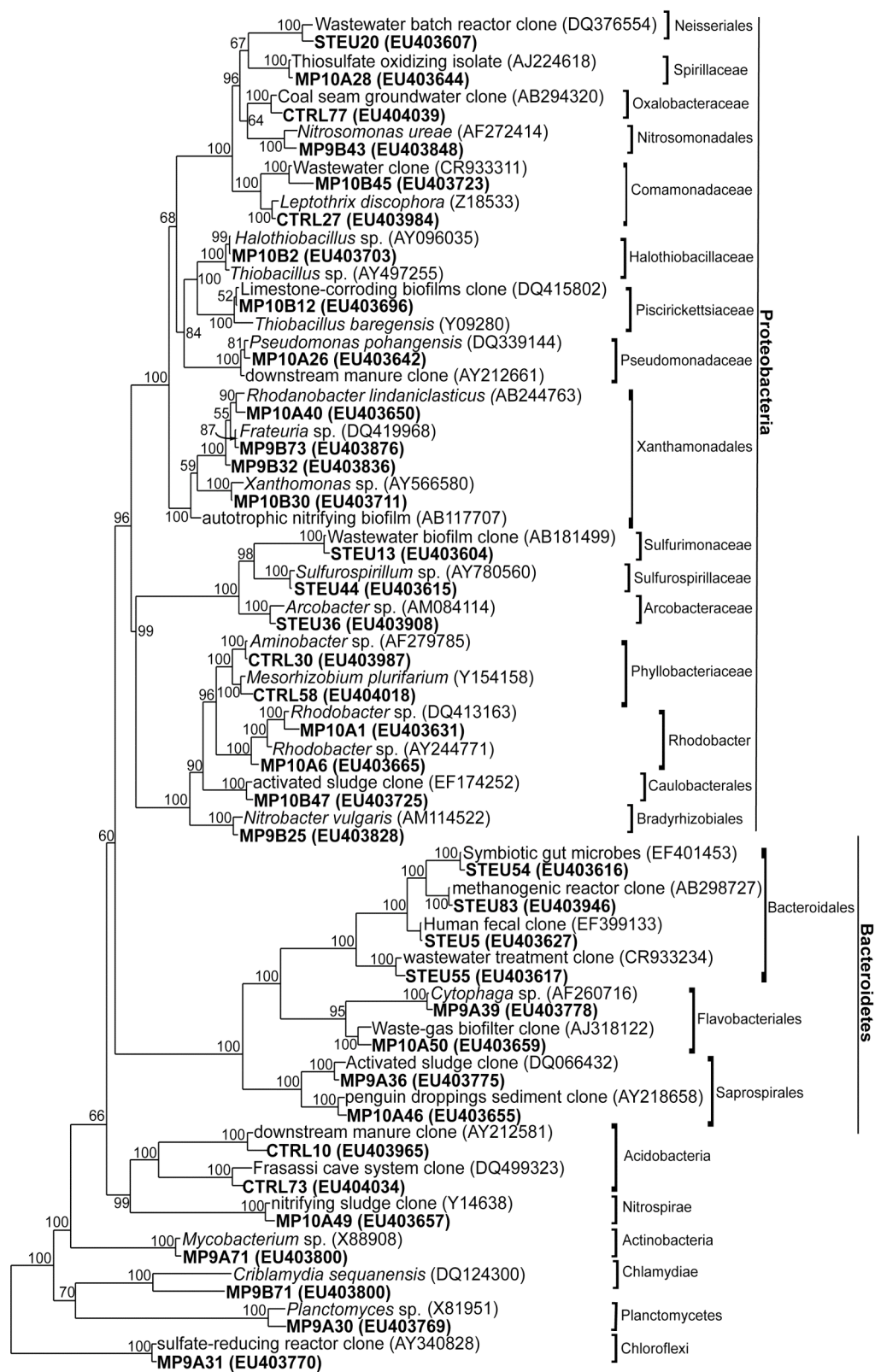


## SUPPLEMENTAL FIGURES



SUPPLEMENTAL. FIG. 1. Environmental scanning electron micrographs at 2000X magnification. (A) open architecture biomat sample taken from a depth of <0.25 cm from test unit receiving STE. Filaments are likely biological and may be EPS or fungal in nature. (B) open architecture infiltrative surface sample taken from a depth of <0.25 cm from test unit receiving clean water.



0.10 substitutions/site

SUPPLEMENTAL FIG. 2. Phylogenetic tree of biomat, control, and STE sequences and relatives. Reference sequences are shown in italics with associated GenBank accession numbers.

SUPPLEMENTAL TABLE 1. Statistical analysis of 16S rRNA gene clone libraries

Sample ID (N=)	Richness	Diversity Indices	
	Rarefaction	Shannon's Index	Simpson's Index
MP10A (62)	36	3.27	0.0402
MP10B (60)	41	3.52	0.0215
MP9A (66)	59	4.03	0.0037
MP9B (77)	56	3.76	0.0266
Control (93)	63	3.98	0.0129
STE (90)	22	2.08	0.2612
Test Unit 10 (122)	73	3.97	0.0219
Test Unit 9 (143)	115	4.60	0.0065
0.5 cm biozone (128)	93	4.32	0.0113
1.0 cm biozone (137)	92	4.18	0.0221
All biozone (256)	180	4.88	0.0096

All statistics are based on 97% sequence identity

## SUPPLEMENTAL METHODS

**Field sampling.** To evaluate the hydraulic and purification processes occurring during onsite wastewater treatment, the Mines Park Test Site was established at the Colorado School of Mines (CSM) in 1998. At this site, STE is obtained from a nearby multifamily apartment building and further treated by different methods such as textile biofilters or membrane bioreactors. Different effluents are applied to replicate soil infiltration cells or drip dispersal lines installed in a native sandy loam soil. Monitoring includes process operation, effluent biogeochemical composition, soil and site properties, and soil pore water composition in the vadose zone (8, 12). As a component of this work, sampling and analysis efforts have enabled detailed microbial characterization to be carried out.

Samples were taken aseptically from the pilot-scale infiltration trenches located at the Mines Park Test Site in Golden, Colorado (17). The microbial communities were analyzed in three different soil treatment units; one (MP10) receiving STE utilized an open architecture above the infiltrative surface (Fig. 1B), another (MP9) utilized a gravel aggregate infiltrative surface (Fig. 1C), and a third soil treatment unit receiving clean tap water utilized an open architecture and served as a negative control. At the time of sampling, the test units had received STE or City of Golden, Colorado tap water (clean water) for 30 months. Two samples were aseptically taken from the top 1.0 cm of infiltrative surface (depth intervals of 0.5 and 1.0 cm), and additional 2.54-cm diameter core samples were taken to a depth of 10 cm, for each test unit receiving STE. For the purposes of this study, the biozone is defined as the top 1.0 cm of infiltrative surface. Another sample was taken from the top 0.5 cm of a test unit receiving clean water as a negative control.

**Culturable enumeration.** Bacteria were extracted from soil by combining approximately 2 g of field moist soil with 20 ml 1.5% (w/v) beef extract solution. The solution was agitated for 30 min at 150 rpm on a rotary wheel, followed by a 15 min settling period. A 5 ml aliquot was taken and serially diluted in phosphate buffered saline (PBS) (15). Diluted samples were analyzed in duplicate for fecal coliforms, heterotrophic plate counts (HPC), and *E. coli*. For fecal coliforms, diluted samples were filtered through a 0.22 µm filter, placed on *m*-FC broth base media (EM Science, San Diego, CA), and incubated at 45°C for 24 hours (1). Fecal coliform bacteria including *E.coli* are members of the total coliform group of bacteria but are characterized by their ability to ferment lactose at 112.1° Fahrenheit (44.5° Celsius) (3). For heterotrophic bacteria, diluted samples were plated on *m*-HPC agar (heterotrophic plate count agar, Becton Dickinson, Franklin Lakes, NJ ) using the spread plate method and incubated at 37°C for 48 hours (1). For *E. coli*, diluted samples were filtered through a 0.22 µm filter, placed on ChromAgar ECC (CHROMagar™, Paris, France ), and incubated at 45°C for 24 hours. Bacterial counts are reported in CFU (colony forming units) per gram dry weight soil.

**Scanning Electron Microscopy (SEM).** In order to determine the visual difference between a soil infiltrative surface dosed with clean water and that of an infiltrative surface with biozone generation, SEM was employed. Infiltrative surface cores were taken to a depth <0.5 cm from a test unit receiving clean water and an open architecture test cell receiving STE. A thin layer that was approximately 0.25 cm thick was aseptically removed from the top of each core for visualization. Images were taken using a Hitachi TM-1000 (Hitachi High-Technologies Corporation Tokyo, Japan) at magnifications from 1000-10,000X.

**Genomic DNA extraction and PCR.** Total DNA was extracted from samples (~400 µg) using the PowerSoil™ DNA Isolation Kit (MO Bio Laboratories, Inc., Carlsbad, CA) per

1 manufacturer's instructions. 16S rRNA genes were amplified from each sample using the  
2 universal primers 515F (5'-GTG CCA GCM GCC GCG GTA A-3') (11) and 1391R (5'-GAC  
3 GGG CGG TGW GTR CA-3') (11). Each 25 µl reaction contained ~100 ng template DNA, 2  
4 mM MgCl<sub>2</sub>, 1X magnesium-free PCR buffer (Promega, Madison, WI), 50 µM of each  
5 deoxynucleoside triphosphate, 200 µM forward and reverse primer, and 1 unit of *Taq*  
6 polymerase. Cycling conditions were 94°C for 2 min; followed by 30 cycles of 94°C for 30 sec,  
7 55.5°C for 1 min, and 72°C for 1.5 min, with a final extension period of 12 min at 72°C.

8 **Cloning and sequencing.** PCR amplicons were gel purified using Montage gel  
9 extraction kit (Millipore Co.) and cloned into TOPO TA pCR<sup>®</sup>4-TOPO<sup>®</sup> (Invitrogen, Carlsbad,  
10 CA), according to manufacturer's instructions. The vector and target sequence were then  
11 transformed into electrocompetent *E. coli* TOP10 cells (Invitrogen, Carlsbad, CA), with a  
12 voltage of 1500 v, a resistance of 125 Ω, and a capacitance of 50 µF. A total of 96 clones were  
13 randomly selected from each sample for sequence analysis. Inserts were amplified by PCR with  
14 the vector specific primers T3 (5'-ATT AAC CCT CAC TAA AGG GA-3') and T7 (5'-TAA  
15 TAC GAC TCA CTA TAG GG-3'). Cycling conditions were 94°C for 2 min; followed by 35  
16 cycles of 94°C for 15 sec, 52°C for 15 sec, and 72°C for 1 min. These amplified clones were  
17 prepared for sequencing with the ExoSAP-it PCR cleanup kit (USB corporation, Cleveland,  
18 OH), and sequenced on a MegaBACE<sup>™</sup> 1000 dye-terminating sequencer (GE Healthcare,  
19 Piscataway, NJ).

20 **Phylogenetic analysis.** 16S sequences were grouped and trimmed with PHRED and  
21 PHRAP (9) in conjunction with XplorSeq (Dr. Daniel Frank, Unpublished). Sequences were  
22 aligned via the NAST aligner at the Joint Genome Institute (<http://greengenes.lbl.gov>), screened  
23 for chimeric sequences with Mallard (2), and manually edited with the ARB phylogenetic

software package (13). Once sequences were aligned, operational taxonomic units (OTUs) were determined by the furthest-neighbor method in DOTUR (16), using a precision of 0.001 and a distance of 0.030. Clones were considered identical if the sequences were  $\geq 97\%$  similar. DOTUR was also used to generate the richness estimators rarefaction, Chao1 (4), and ACE (5, 6) as well as Shannon's and Simpson's diversity indices (14). A total of 447 clones were analyzed for phylogenetic relationships using the Ribosomal Database Project II (RDP) (7).

Selected sequences from this study, including closely related clones from other studies, were exported from ARB using the Lanemask filter. A phylogenetic tree was then calculated with Bayesian inference methods with MrBayes 3.1 (10). Posterior probabilities were converted to percentages at all bifurcations.

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